

Supporting information Fig. 1. Sdc1 expression by thymic *i*NKT cells in NOD and C3H and C57BL/6 mice. Thymocytes were isolated and *i*NKT cells stained and identified using CD1d tetramer gating (left dot plots) and expression of sdc1 by the CD4⁺ and CD4⁻ subpopulations (right dot plots) determined. *i*NKT cells in C3H and NOD, but not C57BL/6 mice, have high frequency of sdc1^{pos} cells, similar to that in Balb/c mice . Data are mean ± s.e.m, n= 2-3 mice per strain.



Supporting information Fig. 2. Detection of sdc1^{pos} *i*NKT subsets in Balb/c mice of increasing age. Flow cytometric analysis of gated *i*NKT cells in thymi of Balb/c mice shows that the frequency (A, B) and absolute number (C) of sdc1^{pos} NKT17 cells peak around 10 weeks of age; n= 3-4 per age group. Error bars indicate SEM. Of note the error bars are too small indicating production of these cells is tightly regulated.



Supporting information Fig. 3. To ensure specificity of sdc1 staining, *I*NKT cells isolated from indicated tissues were stained with loaded CD1d tetramer and TCR β , and PE-conjugated sdc1 specific mAb with PE-conjugated isotype-matched or fluorescence-minus-one (FMO) used as controls. Unloaded tetramer staining was used to gate tetramer+ *I*NKT cells in each tissue (not shown). Dot plots show gating of CD1d tetramer cells (left) and sdc1 expression by gated *I*NKT cells (second left dot plots). Mean expression of sdc1 by gated iNKT cells from three independent experiments for each tissue are shown. It noteworthy that quadrants were set using isotype and FMO for each tissue as indicated. Peripheral lymph node, PLN; white adipose tissue, WAT.



Supporting information Fig. 4. Effect of PMA/ionomycin on surface expression of sdc1 by NKT17 cells. Thymic /NKT cells were sorted from WT mice and cultured in tissue culture medium in the presence or absence of PMA/ionomycin and analyzed after 5 h for sdc1 and IL-17 expression. Dot plots are representative of two experiments with similar results.



Supporting information Fig. 5. Comparison of frequency of TCR β^{hi} and TCR β^{lo} subpopulations of *i*NKT cells in WT and sdc1KO of increasing age. Thymocytes were isolated from age-matched WT and sdc1KO mice and simultaneously analyzed for frequencies of TCR β^{hi} and TCR β^{lo} subpopulations of gated *i*NKT cells. Representative dot plots show gating of TCR β^{hi} and TCR β^{low} subpopulations of *i*NKT cells in specific experiments as different age groups were analyzed in independent experiments on different dates and hence specific gating varied from one experiment to another. Graph shows mean frequency from two independent experiments (n= 4 to 6 mice per genotype per age) are shown. Error bars indicate s.e.m. **P < 0.01 by Mann-Whitney test.



Supporting information Fig. 6. Thymic *I*NKT cells were isolated from age-matched WT and sdc1KO mice and analyzed for intracellular expression of IL-17, IFN- γ and IL-4 by FACS. Error bars indicate s.e.m; n= 3 mice per group. *P <.05 by Mann Whitney test.



Supporting information Fig. 7. Thymic *I*NKT cells were isolated from age-matched WT and sdc1KO mice and analyzed for intracellular expression of PLZF by FACS. Percentage of cells expressing high, medium and low level of PLZF were quantified in individual mice. Mean Values are **from three mice per group**. Error bars indicate s.e.m. *P <.05 by Mann Whitney test.



Supporting information Fig. 8. Serum levels of indicated cytokines in WT and sdc1KO mice at indicated time points after immunization with α GalCer (2 μ g, i.v.). Results expressed as mean \pm s.e.m. are derived from 6 to 8 mice per group. * P < 0.05 by Mann-Whitney test.